PROCESS OPTIMIZATION FOR MANUFRACTURING NON- DAIRY SYNBIOTIC CHOCOLATES AND THEIR NUTRITIONAL CHARACTERIZATION

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DECLARATION

I, **Paromita Mukherjee**, hereby declare that this Synopsis, entitled **"PROCESS OPTIMIZATION FOR MANUFRACTURING NON- DAIRY SYNBIOTIC CHOCOLATES AND THEIR NUTRITIONAL CHARACTERIZATION"** for the registration in Ph.D. Course in Food and Nutrition, was carried out by me.

In course of submission of the Synopsis, I am declaring that this work is an original production of my best endeavor. Any part of this has not been submitted anywhere for any other degree or diploma.

Dated:

PAROMITA MUKHERJEE

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Introduction:

In this modern era, food habits have changed gradually but some foods are gaining attention more and more with new taste, flavor, and texture and also health benefits. The people of all age groups from infants to old age like to consume a food product – chocolate which is being known to all for a long period of time. The chocolate is made generally of ground or roasted kernels of cocoa seeds **(Urbańska and Kowalska, 2019)**. The chocolate can be of various forms such as solid, semi-solid, liquid, pasted and many more **(Rahman and Quodbach, 2021)**. The chocolate is generally sweet in taste in spite of the presence of the bitter cocoa seeds so the flavor found in the chocolate is produced by fermentation process. There are various types of chocolates in the market such as white chocolate, milk chocolate, dark chocolate, baked chocolate etc. **(Shinde and Asrani, 2020)**.

The chocolates which are easily available in the market contains high amount of fat, sugar as well as preservatives thus regular consumption of them can lead to various health problems such as increased blood sugar level, increased cholesterol, and triglyceride levels, causes obesity and may lead to the risk in kidney stone development. Generally, chocolate is made of milk, sugar, cocoa powder, cocoa butter and preservatives **(Konar *et al*., 2023)**.

In this study, a new type of treatment combination will be developed to form a non-dairy synbiotic chocolate in view of deliver a number of health benefits. The newly developed chocolate will have both prebiotic and probiotic characteristics, so it is known as synbiotic **(Ceylan *et al*., 2023)**. Here, the almond skin will act as a prebiotic which have a high dietary fibre content and probiotic effects **(Coutinho *et al*., 2023)**. The almond milk will be used as the substitute for cow's milk. There are numerous benefits of using almond milk on the human health **(Assamoi *et al*., 2023)**. It helps to improve the vision, acts as an anti-obesity agent, helps in bone strengthening and maintains the cardiovascular health **(Rashwan *et al*., 2023)**. It helps muscle development, sustains blood pressure and maintains kidney functions properly.

Almond milk is prepared from soaked almonds which is low in fat content, but energy, proteins, lipids and fiber content is high in it **(De Angelis *et al*., 2023)**. Calcium, magnesium, iron, phosphorus, sodium, potassium and zinc are present in almond milk. Vitamins include such as pyridoxine, vitamin C, thiamine, riboflavin, niacin, folic acid and vitamin E. Almond milk has no cholesterol or lactose in its content so the chocolate made of this can be tolerated by lactose intolerant patient. Almond milk is prepared by grinding the almond seeds along with

water. Due to its higher nutrient content, it is one of the best alternative sources for various diseased patients. Almond milk is mostly considered as safe and affordable as well as it can be included in the diet of vegan people **(Savchuk *et al*., 2015)**.

Here in this new food product development, the Probiotics will act as a probiotic source in this newly developed synbiotic chocolate. Prebiotics and Probiotics has been known to have several health benefits such as proliferating effects on immunological functions, help in digestion and protects human body against various pathogenic bacteria **(Rafique *et al*., 2023)**. So, this newly developed synbiotic chocolate will have various beneficial health effects.

Objective:

1. Optimization of the ratio of prebiotics and probiotics in the newly developed synbiotic chocolate.
2. Evaluation of sensory, physic-chemical and microbiological analysis of synbiotic chocolate.
3. To assess the shelf life (0^{th} to 42^{nd} days) of the newly developed non-dairy synbiotic chocolate at regular interval.
4. Therapeutic effects of newly developed non-dairy synbiotic chocolate analyzing rat model study.
5. Estimation of the cost of newly developed synbiotic chocolate.

Review of Literature:

Chetan *et al*. (2013) revealed that Chocolate is a suspension of fine solid particles of sugar, cocoa and milk powder in a continuous fat phase. Chocolates are solid at ambient (20˚C - 25˚C) and melt at body temperature (37˚C) giving a smooth suspension of particulate solids with a pleasing cooling sensation in the mouth. Probiotic bacteria beneficially affect human health by improving the gut micro biota balance and the defenses against pathogens. Additional health benefits attributed to probiotics are the stimulation of the immune system, blood cholesterol reduction, vitamin synthesis, anti-carcinogenesis and anti-bacterial activities. Two other important criteria to determine the efficacy and the success of the product containing probiotics are the acceptance of the product by the consumers and the survival of probiotic microorganisms during its production

Aspri *et al*. (2020) noted that dairy products have been traditionally considered as the best carriers for probiotic bacteria while in non-dairy probiotic foods have been attracting more attention due to consumer demands. Therefore, in order to alleviate the drawbacks of dairy based probiotics, the development of non-dairy probiotic products, including food matrices from fruit, vegetables, and cereals has a promising future.

Chaluvadi and Bennetzen (2018) reported that Chronic diseases, such as obesity, diabetes and inflammatory bowel disease have a gut microbial component that can be modified by synbiotics supplements or food substances. Including beneficial bacteria and their related products in the diet has potential to stimulate human health and prevent chronic diseases but more research is needed to prove health claims and ensure product safety.

Gadhiya *et al*. (2015) expressed that incorporation of probiotics into chocolate is one of the major challenges to incorporating probiotics into liquid chocolate and chocolate related products on an industrial scale is that these products need to be maintained under specific conditions (like temperature, p^H, etc.) that do not kill the probiotics. This requires a temperature range narrower than that normally used in chocolate manufacturing, making it difficult to achieve effective mixing andproper probiotic dispersion.

Hadi *et al*. (2018) showed that synbiotic supplementation has favorable effect on inflammatory factors, liver enzymes and some anthropometric indices, lipid profiles and glucose homeostasis parameters in patients with Non-Alcoholic Fatty Liver Disease (NAFLD).

Ibrahim *et al*. (2021) studied the impact of lactase from yeasts and molds ingested in the form of capsules and found a resultant significant increase in lactose digestion. *Streptococcus thermophilus* produces β-gal during its transit in the digestive tract, and this enzyme reduces the lactose content. This study was conducted on mice and showed that the produced enzyme is active and able to hydrolyze lactose resulting in an overall reduction of the lactose in feces.

Nobaek *et al.* (2000); Marteau *et al*. (2001) revealed that Probiotics exhibit direct effects in the gut in the treatment of lactose digestion. For example, the administration of the probiotic *Lactobacillus* strain *Lactobacillus planatarum* has been shown to improve lactose digestion, decrease diarrhea and reduce bloating, flatulence and pain during irritable bowel syndrome.

Tremellen & Pearce (2012) said in their study that Probiotics are live micro-organisms. These are synergistic with intestinal microbiota. They may have influence on metabolic and inflammatory conditions. Dairy products including yogurts, fermented foods and some cheeses are known as valuable resources of probiotic cultures in the diet. However, the gut microbiome alteration and biological effects of each source are ambiguous. Available synbiotic supplements are influenced by initial dose strain, quality, temperature and anaerobic storage conditions.

From this review literature, this has been observed that, the previously prepared dairy chocolates are approximately high in refined sugar content and calorie content. So, this research will be serving as a bridge between the conventional dairy chocolates and non-dairy chocolates, enriched with synbiotics that can serve a large variety of health conditions like lactose intolerance, celiac disorders, diabetes mellitus and many more.

Plan of work:

1ST YEAR – Chocolate is a food product which can be consumed by the people of any age group due to high sugar, fat and preservative content, the high consumption of it can leads to various disease conditions such as obesity, high cholesterol level, diabetes etc. here in this study, the focus will be to develop a new synbiotic chocolate with high amount of beneficial nutrients as well as health benefits. Here the oats powder will be act as a prebiotic to make this newly developed chocolate. The oats powder contains high amount of dietary fibre, rich with antioxidant activity and affordable in price. This will be added along with extracted Streptococcus thermophilus strain of bacteria which will act as a probiotics. This prebiotic and probiotic will be added to the newly formed treatment combination to prepare this synbiotic chocolate. Nine treatment combinations will be developed for prebiotic and probiotic addition and one control will also be formed. The newly formed synbiotic chocolate will then undergo the sensory evaluation with the help of the hedonic scale rating. The color, flavor, texture and taste will be assessed for the acceptance of newly developed synbiotic chocolate. Next, the physic-chemical analysis will be done to analyze the pH of the synbiotic chocolate as well as the dietary fibre, fat, protein, carbohydrate content is evaluated. The antioxidant activity of the newly developed synbiotic chocolate will also be estimated. The synbiotic chocolate will be prepared for the microbiological analysis after that.

2ND YEAR – The newly developed synbiotic chocolate will then be microbiologically analyzed. The pathogen if present will be detected and the final food product will be pathogen free. The tests will be done to detect the presence of yeast and mold as well as Escherichia Coli bacteria. The Standard Plate Count (SPC) will be done to detect the presence the bacterial growth. Then the Prebiotic Index test will be done to analyze the activity of prebiotics added in the newly developed synbiotic chocolate. The viability test will also be done to detect the activity of probiotics in synbiotic chocolate along with study of shelf life of the developed food product. After that, the therapeutic benefits will be analyzed using rat model study.

3RD YEAR – The final year is the most crucial one. The cost analysis will be done for one chocolate so that the difference from the market available chocolate can be scanned and the cost of newly developed synbiotic chocolate can be established. Also, the publications of the recent work have to be done. The data which have been collected in the first and second year, they have to be analyzed by the using of various computerized statistical tools. This

novel work has to be submitted for patent filing. The thesis writing has to be started in the beginning of the third year and have to be submitted by the end of the third year.

Methodology:

The research, entitled "**Process optimization for manufacturing synbiotic non-dairy chocolate and their nutritional characterization**" will be carried out in the Food and Nutrition Laboratory, Swami Vivekananda University, Barrackpore.

Procurement of Raw Material

For preparation of control and experimental synbiotic non-dairy chocolate, the raw ingredients like Cocoa butter, Cocoa powder, Icing sugar, soy lecithin, almonds, oats, coconut oil, beetroot will be bought from local market.

Cocoa Butter

Pure & raw Cocoa butter will be bought from local market of Dunlop.

Cocoa Powder

Natural cocoa powder will be collected from local shop of Dunlop.

Sugar

Commercial grade clean, white crystalline sugar will be obtained from the local shop of Dunlop.

Stabilizers and emulsifier

Soy Lecithin will be bought from local shop of dunlop.

Almonds:

Sweet almonds (P. dulcis) will be bought from locals market.

Oats:

Ground oats will be collected from local grocery stores of Dunlop.

Edible Coconut Oil:

Edible coconut oil will be collected from local markets of baranagar.

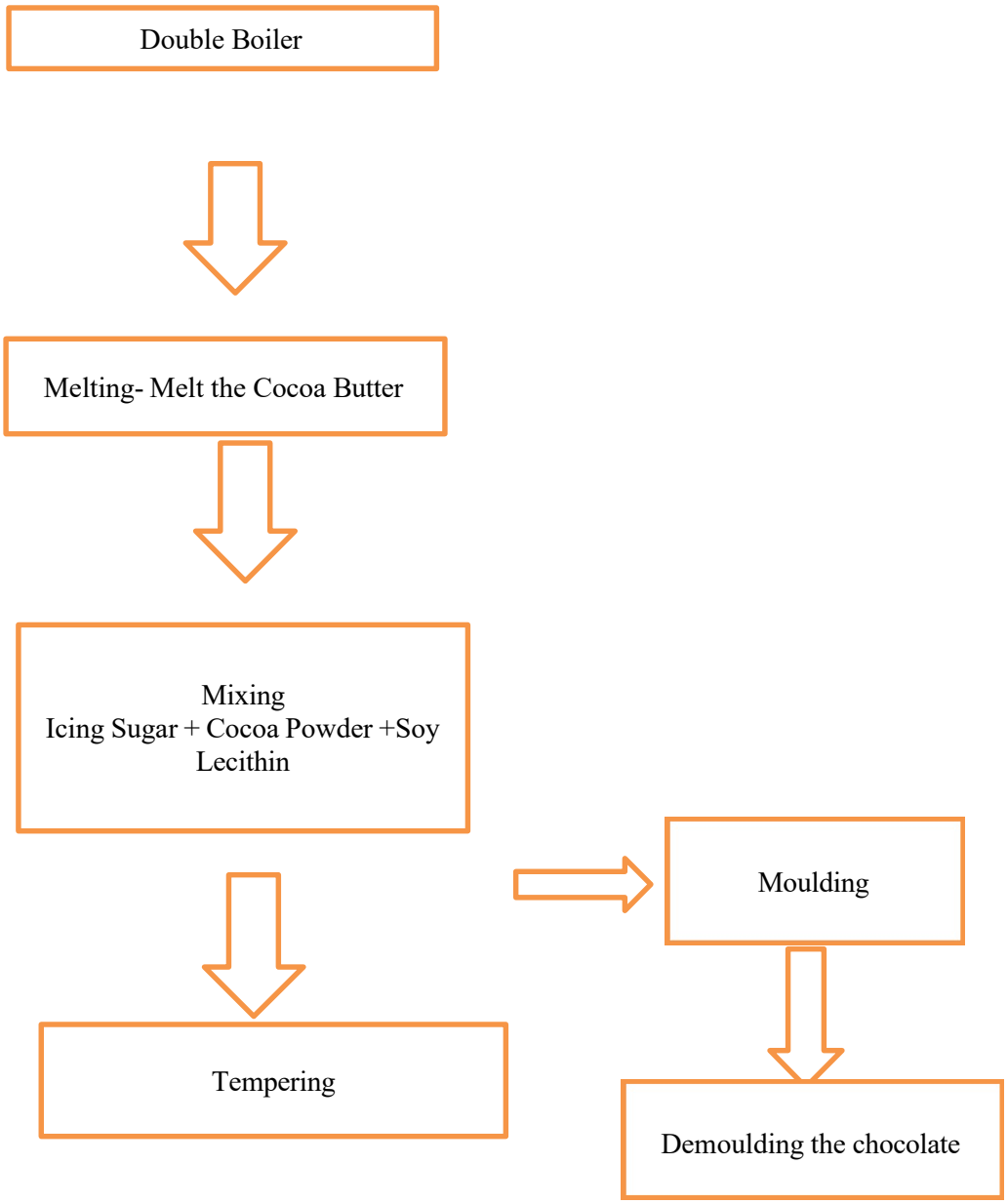
Soy Lecithin:

Soy lecithin will be purchased from E-Commerce shops.

Beetroot:

Beetroot will be purchased from locals sabji mundi of baranagar.

Flowchart for preparation of Control Chocolate (Yates, 2009).



Treatment Combinations of control and experimental chocolates:

Following are the treatment combinations (T_1, T_2, T_3 and T_4) for experimental chocolates:

T0 - Cocoa powder +Dry Milk powder + Cocoa butter + Sugar

1. T_1 - Almond milk (60ml) + Oats (30gms) + Coconut oil (5ml) + Soya lecithin (0.5gms) +Beet root powder (4.5 gram) +1% probiotics culture
2. T_2 - Almond milk (65ml) + Oats (25gms) + Coconut oil (5ml) + Soya lecithin (0.5gms) +Beet root powder (4.5 gram) +1% probiotics culture
3. T_3 - Almond milk (70ml) + Oats (20gms) + Coconut oil (5ml) + Soya lecithin (0.5gms) +Beet root powder (4.5 gram) +1% probiotics culture
4. T_4 - Almond milk (75ml) + Oats (15gms) + Coconut oil (5ml) + Soya lecithin (0.5gms) +Beet root powder (4.5 gram) +1% probiotics culture.

The experimental chocolates will be followed by, Sensory evaluation, physicochemical analysis and Microbiological studies.

Sensory evaluation of experimental chocolate:

There are numerous, diverse tests that can be used for the sensory evaluation of chocolate products. The appropriateness depends on the question of interest. Those are namely, Threshold Test, Discrimination Test, Hedonic Tests (Harwood et al., 2017).

Threshold Tests:

Threshold tests are a class of methods that attempt to estimate the point at which a stimulus, or a change in stimulus, becomes perceptible. The detection threshold estimates the minimal physical stimulus that an observer can detect, irrespective of quality; this is sometimes called the absolute threshold thresholds can be determined for individual observer (requiring many trials) or for a group of observers (Manthilal et al., 2019). Moreover, it is important to consider that threshold tests are generally carried out in the simplest of models systems and this becomes a much more complicated task when carried out in a complex stimuli such as chocolate. Threshold test implies sensory acuity of individuals who are being considered for trained panels or other tasting roles (Pieracci et al., 2021).

Discrimination Test:

Discrimination test is described as any method to determine if differences among stimuli are perceptible. This is particularly concerning when dealing with a complex product such as chocolate, where the increasing complexity of the product is generally accompanied by an increase in noise in the samples, making true differences more difficult to detect. the sensory scientist must consider the relative signal to noise ratio for the specific product when determining the appropriate sample size and size of the sample (Harwood et al., 2017).

Hedonic Test:

Hedonic testing can be a useful tool for understanding how consumers perceive and react to specific products affectively. Liking, preference and/or acceptability can all be measured, both for products as a whole and for specific attributes. There is an abundance of tools that can be used to accomplish these types of goals. For acceptability and appropriateness, common tools could include scales, such as the nine-point hedonic scale (Wichchukit, & O'Mahony, 2015). Over the last half century a number of scales have been developed and utilized to measure hedonic responses in both basic psychophysical and applied research. Some of the hedonic scaling methods will be discussed below together with each scale's utilities, properties, advantages, and disadvantages (Lim, 2011).

Physicochemical analysis of experimental chocolate:

The following physicochemical analysis will be carried out for the experimental chocolates.

Those are as follows:

Texture Analysis

Texture will be determined using Texture Analyser. The sample will be kept on the flat platform of the instrument and was subjected to double compression by a cylindrical probe with 5mm diameter. The test will be conductedat a speed of 10 mm/s using 50 N load cell.The sample will be allowed for double compression of 40% with trigger force of 0.5kg during which various textural parameters were determined from the force deformation curve, the hardness, cohesiveness, adhesiveness and gumminess were determined **(Muhammad *et al*., 2018)**

Moisture

Moisture content of chocolates will be estimated by the method of A.O.A.C. To determine the moisture content of the sample, ten grams of the sample was takenin a petridish and dried in a hot air oven at 60^0C to 70^0C, cooled in a desicator and weighed. The process of heating and cooling will be repeated till constant weight was achieved. The moisture content of thesample will be calculated from the loss inweight during drying **(Acan *et al*., 2021)**

Energy

Energy content of selected experimental chocolates will be calculated according to Gopalan *et al* and expressed as kilocalories (kcal). The energy present in sample was calculated asper the formula given below:

Energy = (4 x Protein) + (4 x Total carbohydrates) +(9 x Fat) (Faccinetto-Beltrán *et al*., 2021)

Reducing sugar

Twenty-five gram of chocolates will be ground with 100 ml of distilled water and transferred to a conical flask. It was neutralized with 1 N sodium hydroxide in the presence of phenolphthalein. For the clarification of the neutralized mixture, 2 ml of lead acetate will be added followed by addition of 2 ml of potassium oxalate to neutralize the excess amount of lead acetate. The mixture will then allow to stand for 10minutes for the settlement of the precipitate. The solution will be filtered through filter paper. The solution will be titrated against a boiling mixture of Fehlings solution A and B using methylene blue as anindicator until the appearance of brick red colour indicator **(Shahanas *et al*., 2020)**.

Protein estimation

The protein content of chocolates will be estimated using Lowry's method given by Sadasivam and Manickam. A sample of 500 mg will be extracted using 5 to10 ml of buffer (Tris buffer GR- trishydroxyl methylaminomethane) and centrifuged **(Hadnađev *et al*., 2023)**.

Total fat Estimation

Ten grams of chocolates was wrapped in a blotting paperand tied with twine. The sample is placed in the extraction tube of Soxhlet apparatus. The fat present in the chocolates was extracted through siphoning of petroleum ether through the apparatus and fat will getsettled at

the bottom of the flask along withlittle amount of petroleum ether. This was transferred to a pre-weighed beaker and kept open for the petroleum ether to evaporate. The cream-coloured substancesleft behind after the evaporation of solvent was the fat and it was weighed and expressed as percentage **(Foong *et al*., 2013)**.

Ash content

The ash content of the chocolate's estimation will be done by using the method given by ISI. Five grams of sample will be taken in a crucible and was ignited at 550-600°C in a muffle furnace for 5-6 hours. Cooled in desiccator at room temperature and weighed. The ash content of sample was expressed in percentage **(Foong *et al*., 2013)**.

Microbiological studies of experimental chocolates:

The growth media, will be used for the microbiological enumeration of the different microorganisms were: Plate Count Agar (PCA), MacConkey Agar (for Escherichia coli), Salmonella-Shigella Agar, Bairdparker Agar (for Staphylococcus aureus) and Czapek Agar (for yeasts and molds). For each test, 3 g of ground sample will be homogenized in 30 ml of corresponding broth in sterile flask and incubated for 24 h at 37 °C. Samples (1 ml) will be aseptically removed and progressive decimal dilutions (101 - 108) will be prepared in water peptone, mixed using a vortex for a few seconds and plated onto appropriate media. The inoculated plates will be incubated for 48 h at 37 °C except for yeasts and molds determination for 5 d at 25 °C and the colonies were counted manually using an impermanent marker **(Gounga *et al*., 2008)**.

Probiotic Viability Test:

The probiotic viability can be assessed during the entire storage period of 120 days at 30 days interval. Samples will be mixed with 225 mL of sterile 0.1% peptone water and serially diluted, plated on MRS selective medium and incubated anaerobically at 37 °C for 48 h **(Santos *et al*., 2016)**.

Laličić-Petronijević *et al*. (2015) revealed in their study that survival of probiotic strains L. acidophilus in various types of chocolate (milk and dark with 75% cocoa parts) was generally very good. During the entire storage period of 180 days the number of viable cells of mentioned strain remained at the level of 8 log cfu/g, or, with a small number of samples, 7 log cfu/g, at

both investigated temperatures. The probiotics applied can maintain higher viability for up to 180 days of storage at 4 °C **(Hossain *et al*., 2021)**.

Prebiotic Index:

Prebiotic index will be calculated by the ratio of probiotic growth in the prebiotic to probiotic growth in a control carbohydrate. A prebiotic index higher than 1 means that the carbohydrate has a positive effect on the probiotic growth. If the prebiotic index is near to 1, indicates a low effectiveness of the evaluated carbohydrate. The prebiotic index was calculated according to equation:

Prebiotic Index (Ipreb) = CFU of probiotics in prebiotic carbohydrate /CFU of probiotics in control carbohydrate **(Figueroa-Gonzalez *et al*., 2019)**.

Substrates with a high prebiotic activity score support good growth of probiotic bacteria, with cell counts (CFU/mL) comparable with that when grown on lactose **(Homayouni & Rasouli Pirouzian, 2019)**.

Rat Model Study:

Rat model study will be carried out for evaluating the therapeutic potential of the newly developed non-dairy synbiotic chocolate on the upper gastrointestinal tract, including the stomach, liver, and small intestine, using a Wistar albino rat model. Experimental groups included a control group, a diseased group will induce with irritable bowel syndrome (IBS) via Dextrose Sulfate Sodium (DSS), and a treatment group which is subjected to 30 days of synbiotic chocolate administration (Mahoro et al., 2021). The synbiotic chocolate formulation comprised almond milk (65%), oats powder (25%), beetroot powder (5%), *Streptococcus thermophilus* (1%), and coconut oil (4%).

Significance:

- This study will focus on the development of synbiotic chocolate with the combination of prebiotics and probiotics. The almond skin with high dietary fibre and antioxidant activity will act as prebiotic and the *Streptococcus thermophilus (BURD PB)* will act as a probiotic to make a healthy and nutritious synbiotic chocolate.
- The sensory, physico-chemical and microbiological analysis of the newly developed synbiotic chocolate will be helpful to evaluate the acceptance of the synbiotic chocolate in the society as well as to the people suffering from various disease conditions.

- The rat model study will be helpful to evaluate the therapeutic potential of the newly developed non-dairy synbiotic chocolate on the upper gastrointestinal tract, including the stomach, liver, and small intestine which may suggest the synbiotic chocolate formulation can effectively promotes gastrointestinal health
- This study will help to develop a low cost as well as affordable synbiotic chocolate with high nutrient content which will be especially beneficial to human health.

Possible Outcomes:

1. The newly developed synbiotic chocolate will be formulated with the standardized ratio of prebiotics and probiotics.
2. A highly nutritious synbiotic chocolate with high dietary fibre, low fat and low sugar content as well as high antioxidant activity.
3. The developed synbiotic chocolate will be beneficial for promoting gastrointestinal system.
4. A low cost synbiotic chocolate will be developed with numerous health benefits.

References:

- Acan, B. G., Toker, O. S., Palabiyik, I., Pirouzian, H. R., Bursa, K., Kilicli, M., ... & Konar, N. (2021). Physicochemical properties of chocolate spread with hazelnut cake: Comparative study and optimization. *LWT*, *147*, 111548.
- Aspri, M., Papademas, P., & Tsaltas, D. (2020). Review on non-dairy probiotics and their use in non-dairy based products. *Fermentation*, *6*(1), 30.
- Assamoi, A. A., Atobla, K., Ouattara, D. H., & Koné, R. T. (2023). Potential Probiotic Tiger Nut-Cashew Nut-Milk Production by Fermentation with Two Lactic Bacteria Isolated from Ivorian Staple Foods. *Agricultural Sciences*, *14*(4), 584-600.
- Ceylan, H. G., & Atasoy, A. F. (2023). New Bioactive Edible Packing Systems: Synbiotic Edible Films/Coatings as Carries of Probiotics and Prebiotics. *Food and Bioprocess Technology*, 1-16.
- Chaluvadi, S., & Bennetzen, J. L. (2018). Species-associated differences in the below-ground microbiomes of wild and domesticated Setaria. *Frontiers in plant science*, *9*, 1183.
- Chetana, R., Reddy, S. R. Y., & Negi, P. S. (2013). Preparation and properties of probiotic chocolates using yoghurt powder. *Food and nutrition sciences*, *4*(3), 276-281.

- Coutinho, G. S. M., Ribeiro, A. E. C., Prado, P. M. C., Oliveira, É. R., Careli-Gondim, Í., Oliveira, A. R., ... & Vilas Boas, E. V. D. B. (2023). New plant-based fermented beverage made of baru nut enriched with probiotics and green banana: composition, physicochemical and sensory properties. *Journal of Food Science and Technology*, 1-12.
- De Angelis, D., Pasqualone, A., Squeo, G., & Summo, C. (2023). Almond okara as a valuable ingredient in biscuit preparation. *Journal of the Science of Food and Agriculture*, *103*(4), 1676-1683.
- Faccinetto-Beltrán, P., Gómez-Fernández, A. R., Orozco-Sánchez, N. E., Pérez-Carrillo, E., Marín-Obispo, L. M., Hernández-Brenes, C., ... & Jacobo-Velázquez, D. A. (2021). Physicochemical properties and sensory acceptability of a next-generation functional chocolate added with omega-3 polyunsaturated fatty acids and probiotics. *Foods*, *10*(2), 333.
- Foong, Y. J., Lee, S. T., Ramli, N., Tan, Y. N., & Ayob, M. K. (2013). Incorporation of Potential Probiotic L actobacillus plantarum Isolated from Fermented Cocoa Beans into Dark Chocolate: Bacterial Viability and Physicochemical Properties Analysis. *Journal of Food Quality*, *36*(3), 164-171.
- Gadhiya, D., Patel, A., & Prajapati, J. B. (2015). Current trend and future prospective of functional probiotic milk chocolates and related products-a review. Czech Journal of Food Sciences, 33(4), 295-301.
- Gounga, M. E., Xu, S. Y., & Wang, Z. (2008). Nutritional and microbiological evaluations of chocolate-coated Chinese chestnut (Castanea mollissima) fruit for commercial use. *Journal of Zhejiang University Science B*, *9*, 675-683
- Hadi *et al*. (2018) showed that synbiotic supplementation has favorable effect on inflammatory factors, liver enzymes and some anthropometric indices, lipid profiles and glucose homeostasis parameters in patients with NAFLD.
- Hadnađev, M., Kalić, M., Krstonošić, V., Jovanović-Lješković, N., Erceg, T., Škrobot, D., & Dapčević-Hadnađev, T. (2023). Fortification of chocolate with microencapsulated fish oil: Effect of protein wall material on physicochemical properties of microcapsules and chocolate matrix. *Food Chemistry: X*, *17*, 100583.
- Harwood, M. L., & Hayes, J. E. (2017). Sensory evaluation of chocolate and cocoa products. *Beckett's Industrial Chocolate Manufacture and Use*, 509-520.
- Hossain, M. N., Ranadheera, C. S., Fang, Z., & Ajlouni, S. (2021). Impact of encapsulating probiotics with cocoa powder on the viability of probiotics during chocolate processing, storage, and in vitro gastrointestinal digestion. *Journal of Food*

Science, *86*(5), 1629-1641.

- Ibrahim, S. A., Gyawali, R., Awaisheh, S. S., Ayivi, R. D., Silva, R. C., Subedi, K., ... & Krastanov, A. (2021). Fermented foods and probiotics: An approach to lactose intolerance. *Journal of Dairy Research*, *88*(3), 357-365.
- Konar, N., Polat, D. G., Dalabasmaz, S., Erdogan, M., Sener, S., & Sarıkaya, E. K. (2023). Effects of various milk powders on main quality parameters of cocoa butter substitute-based chocolate. *International Dairy Journal*, *139*, 105571.
- Laličić-Petronijević, J., Popov-Raljić, J., Obradović, D., Radulović, Z., Paunović, D., Petrušić, M., & Pezo, L. (2015). Viability of probiotic strains Lactobacillus acidophilus NCFM® and Bifidobacterium lactis HN019 and their impact on sensory and rheological properties of milk and dark chocolates during storage for 180 days. *Journal of Functional Foods*, *15*, 541-550.
- Lim, J. (2011). Hedonic scaling: A review of methods and theory. *Food quality and preference*, *22*(8), 733-747.
- Mahoro, P., Moon, H. J., Yang, H. J., Kim, K. A., & Cha, Y. S. (2021). Protective effect of gochujang on inflammation in a DSS-induced colitis rat model. *Foods*, *10*(5), 1072.
- Mantihal, S., Prakash, S., & Bhandari, B. (2019). Texture-modified 3D printed dark chocolate: Sensory evaluation and consumer perception study. *Journal of texture studies*, *50*(5), 386-399.
- Marteau, P. R., Vrese, M. D., Cellier, C. J., & Schrezenmeir, J. (2001). Protection from gastrointestinal diseases with the use of probiotics. *The American journal of clinical nutrition*, *73*(2), 430s-436s.

- Muhammad, D. R. A., Saputro, A. D., Rottiers, H., Van de Walle, D., & Dewettinck, K. (2018). Physicochemical properties and antioxidant activities of chocolates enriched with engineered cinnamon nanoparticles. *European Food Research and Technology*, *244*, 1185-1202.
- Nobaek, S., Johansson, M. L., Molin, G., Ahrné, S., & Jeppsson, B. (2000). Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *The American journal ofgastroenterology*, *95*(5), 1231-1238.
- Pieracci, Y., Ascrizzi, R., Pistelli, L., & Flamini, G. (2021). Comparison of the chemical and sensorial evaluation of dark chocolate bars. *Applied Sciences*, *11*(21), 9964.
- Rafique, N., Jan, S. Y., Dar, A. H., Dash, K. K., Sarkar, A., Shams, R., ... & Hussain,
- Rahman, J., & Quodbach, J. (2021). Versatility on demand–The case for semi-solid micro-extrusion in pharmaceutics. *Advanced Drug Delivery Reviews*, *172*, 104-126.
- Rashwan, A. K., Osman, A. I., & Chen, W. (2023). Natural nutraceuticals for enhancing yogurt properties: a review. *Environmental Chemistry Letters*, *21*(3), 1907-1931.

  S. Z. (2023). Promising bioactivities of postbiotics: A comprehensive review. *Journal of Agriculture and Food Research*, 100708.
- Santos, T. T., Ornellas, R. M. S., Arcucio, L. B., Oliveira, M. M., Nicoli, J. R., Dias, C. V., ... & Vinderola, G. (2016). Characterization of lactobacilli strains derived from cocoa fermentation in the south of Bahia for the development of probiotic cultures. *LWT*, *73*, 259-266.
- Savchuk, Y., Usatyuk, S., & Smirnova, E. (2015). *Health benefits of almond milk* (Doctoral dissertation).
- Shahanas, E., Panjikkaran, S. T., Sharon, C. L., Aneena, E. R., Suma, B., Minimol, J. S., & Remya, P. R. (2020). Physico Chemical Properties of Chocolates and its Variability with Process Conditions. *Indian Journal of Nutrition and Dietetics*, *57*(1), 73.
- Shinde, Y., & Asrani, R. (2020). Proximate Analysis of Chocolates Available in Indian Market. *International Journal of Applied Chemical and Biological Sciences*, *1*(2), 26-36.
- Tremellen, K., & Pearce, K. (2012). Dysbiosis of Gut Microbiota (DOGMA)–a novel theory for the development of Polycystic Ovarian Syndrome. Medicalhypotheses, 79(1), 104-112.
- Urbańska, B., & Kowalska, J. (2019). Comparison of the total polyphenol content and

antioxidant activity of chocolate obtained from roasted and unroasted cocoa beans from different regions of the world. *Antioxidants*, *8*(8), 283.

- Wichchukit, S., & O'Mahony, M. (2015). The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives. *Journal of the Science of Food and Agriculture*, *95*(11), 2167-2178.